

GAO Audit on Alternatives to Animal Research: ORD Responses to GAO Follow-up Items

This document provides responses to the GAO Audit on Alternatives to Animal Research (Job Code# 102874), specifically follow up information resulting from the GAO meeting with ORD subject experts on Oct 24, 2018. The written responses are accompanied by three supporting documents: 1) FY13-14 Report to Congress on ToxCast Activities; 2) EPA's Laboratory Animal Project Review Protocol; and 3) ORD-NCCT's Cooperative Research and Development Agreement (CRADA) with L'Oréal on cosmetic testing alternatives.

Responses to GAO Follow-up Items:

1. Actual numbers of animals used in projects and number of those projects in NRMRL, NHEERL, and NERL from 2016-2018.

ORD Response: The National Health and Environmental Effects Research Laboratory (NHEERL) Research Triangle Park (RTP) facility currently has 21 Principal Investigators conducting research using animal models with a total of 53 approved and active Laboratory Animal Project Reviews. NHEERL animal use at the RTP facility over the past three years is detailed in Table 1.

Table 1. NHEERL RTP Animal Use 2016-2018				
Year	Mice	Rat	Rabbit ¹	Zebrafish ²
2016	2716	9037	10	45,453
2017	2472	5024	8	26,730
2018	1580	3791	6	29,649

¹ A large percentage of the rats and mice, and all the rabbits, were used to generate cells for primary cell cultures, another alternative to whole animal research.

² Majority of the zebrafish were used in larval stages, which are not considered vertebrate animals.

NHEERL also has four ecology divisions located in Gulf Breeze FL (Gulf Ecology Division, GED), Corvallis OR (Western Ecology Division, WED), Duluth MN (Mid-Continent Ecology Division, MED), and Narragansett RI (Atlantic Ecology Division, AED). Of those, only GED, MED and AED conduct research using animal models and all of those are aquatic species. NHEERL is finalizing formal IACUCs at these facilities

NHEERL/AED has two active research protocols using the Killifish (*Fundulus heteroclitus*). These fish are caught in the wild and held in the laboratory for breeding stock or used directly in on-going research. Killifish use by AED for the period 2016 to 2018 is summarized in Table 2.

Table 2. NHEERL AED Animal Use 2016-2018	
Year	Killifish (<i>Fundulus heteroclitus</i>)
2016	2000
2017	2000
2018	3000

NHEERL/GED has two active research protocols using multiple species of aquatic animals. These animals are reared from eggs and then some of the animals are used for research. The total number of animals by GED for the period 2016 to 2018 is summarized in Table 3.

Table 3. NHEERL GED Animal Use 2016-2018							
Year	African Clawed Frog	Southern Toad	Southern Leopard Frog	Bronze Frog	Zebrafish	Fathead Minnow	Sheepshead Minnow
2016	3322	155	0	0	400	0	0
2017	5336	171	59	0	0	865	103
2018	7185	1	1000	1	0	400	0

NHEERL/MED has seven active research protocols using multiple species of aquatic animals at various life-stages. The total number of animals used by MED for the period 2016 to 2018 is summarized in Table 4.

Table 4. NHEERL MED Animal Use 2016-2018							
Year	African Clawed Frog¹	Rainbow Trout²	Japanese Medaka³	Zebrafish⁴	Mosquito Fish⁵	Fathead Minnow⁶	Other Fish⁷
2016	480	528	2460	0	0	1726	0
2017	480	528	504	432	0	3080	100
2018	830	663	0	120	316	4170	0

¹ Eggs and/or tadpoles

² Juvenile and adult fish

³ Eggs to adults

⁴ Larvae to adults

⁵ Adults

⁶ Eggs to adults

⁷ Adults

The National Exposure Research Laboratory (NERL) and National Risk Management Research Laboratory (NRMRL) located in Cincinnati OH share a single IACUC. Combined, they currently have 6 Principle Investigators conducting research using animals with a total of 13 approved and active Laboratory Animal Project Reviews. Animal use at the Cincinnati facility over the past three years is detailed in Table 5.

Table 5. NERL and NRMRL Cincinnati Animal Use 2016-2018						
Year	Mice	Chicken	Duck	Fathead Minnow	Zebrafish	
2016	387	330	58	50,048	0	
2017	350	284	41	30,200	200	
2018	419	0	0	7,000	1,000	

- Information on ORD's Institutional Animal Care and Use Committees (IACUC) structure. List of the locations, contact person, and the scope, including which labs are responsible for each of the 5 IACUCs.

ORD Response: There are 3 additional research laboratories in NHEERL which use vertebrate animals. These laboratories are located as follows:

- Atlantic Ecology Division (AED), Narragansett, RI
- Gulf Ecology Division (GED), Gulf Breeze, FL
- Mid-Continent Ecology Division (MED), Duluth, MN

All three of the Ecology Divisions work only with aquatic species; vertebrates are limited to fish and amphibians. The Ecology Divisions do not have a regulatory reporting requirement (no USDA species, no Public Health Service (PHS) assurance) and as such do not track their animal use numbers, nor do they maintain databases of approved protocols.

All the ORD IACUC currently, or shortly will, consist of the following minimum 5 members:

- IACUC Chair
- Attending Veterinarian (AV): A licensed veterinarian with experience in the laboratory animal models used.
- Scientific Member: A scientist with experience in animal research. The ORD IACUCs each have multiple scientific members.
- Non-Scientist
- Non-Affiliated member.

Per Office of Laboratory Animal Welfare (OLAW) policy, the non-scientist and non-affiliated members of the IACUC can be the same individual, which is what is in place at the PHS-assured NHEERL RTP facility.

Contact information for the IACUC Chair is as follows:

- Cincinnati IACUC: Michael Ware (ware.michael@epa.gov; 513-569-7731),
- NHEERL RTP IACUC: Jaimie Graff (graff.jaimie@epa.gov; 919-541-0690)
- Ecology Division IACUCs: Jaimie Graff can also answer questions regarding the IACUC being constituted at NHEERL's other facilities.

3. [Animal Care and Use protocol document.](#)

ORD Response: See attachment 3. LAPR.pdf. (Laboratory Animal Project Review template)

4. [Links to websites/documentation for STAR grant solicitations that are specific to developing an alternative approach to animal research.](#)

ORD Response:

- a) Organotypic Culture Models (OCM)s for Predictive Toxicology Center
(https://cfpub.epa.gov/ncer/abstracts/index.cfm/fuseaction/display.rfatext/rfa_id/577)
- b) Development and Use of Adverse Outcome Pathways that Predict Adverse Developmental Neurotoxicity
(https://cfpub.epa.gov/ncer/abstracts/index.cfm/fuseaction/display.rfatext/rfa_id/562)
- c) Advancing Actionable Alternatives to Vertebrate Animal Testing for Chemical Safety Assessment
(https://cfpub.epa.gov/ncer/abstracts/index.cfm/fuseaction/display.rfatext/rfa_id/642)

5. [STAR grant successes in supporting CSS program and any other successes related to alternatives to animal research.](#)

ORD Response: The following are examples of STAR grant successes in support promoting the public purpose of advancing alternatives to animal testing. STAR research centers are developing cell models for high priority biological systems such as brain, liver, heart and kidney to accelerate research on the interactions of chemicals with key biological processes. In

addition, STAR researchers work directly with EPA/ORD/CSS scientists to implement quantitative risk assessment techniques that reduce uncertainties in extrapolation from *in vitro* systems to *in vivo* outcomes. STAR/CSS researchers have published hundreds of peer review scientific reports, many of them coauthored by STAR researchers and ORD scientists. These reports have been published in high quality journals and describe, for example, methods and models for predictive toxicology, which are directly relevant to the objectives of the CSS program. Examples of these publications can be found at NCER internet page:

<https://www.epa.gov/research-grants/safer-chemicals-research-grants>.

6. Examples of most significant recent or ongoing efforts to conduct intramural research on the development of alternative methods; how success is measured; and links to online tools and information.

ORD Response: Ongoing EPA research is focused on developing and applying new approach methods to support to TSCA and EDSP. These methods will be used for chemical prioritization, support for data gap-filling approaches (e.g., chemical read-across), and informing the identification of the most appropriate animal testing (leading to reduced animal testing). Below we have described in more detail how EPA's new approach methods are currently supporting EDSP and TSCA. In addition, we have summarized new approaches that are currently under development and not yet ready for use in regulatory decisions.

Supporting EPA Decisions

- ORD is working closely with EPA's Toxics Program to develop and use new approaches for the implementation of the Toxic Substances Control Act (TSCA) as amended by the Frank R. Lautenberg Chemical Safety for the 21st Century Act. EPA's Toxics Office (OPPT) is responsible for carrying out the mandates of TSCA which include new requirements and deadlines for actions related to the assessment and regulation of new and existing chemical substances. Under the amended TSCA, EPA has developed a Strategic Plan to describe a multi-year process with incremental steps for adoption and integration of appropriate and fit-for-purpose new approach methods with other alternative approaches for making TSCA decisions (e.g., prioritization, risk evaluations and other risk-based decisions)¹. The 2018 *Strategic Plan to Promote the Development and Implementation of Alternative Test Methods Within the TSCA Program* describes EPA's approach for using these new methods. EPA's long-term goal is to move towards making TSCA decisions with new approach methods (NAMs) to reduce and eventually eliminate vertebrate animal testing for TSCA. Following public comment of the working approach released on September 28, 2018, the proposed binning approach will be piloted in a proof-of-concept white paper that will be released for stakeholder input and public comment and discussed at a public meeting.

¹ Published on June 22, 2018 at <https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/strategic-plan-reduce-use-vertebrate-animals-chemical>

- ORD is working with EPA's Endocrine Disruption Screening Program to develop and use new approaches to assess chemical disruption of estrogen, androgen, and thyroid hormone signaling, as well as steroidogenesis². The estrogen model was completed first and is replacing the previous tier 1 testing that depended on animals. The androgen model is close to being implemented, although more research is needed. The thyroid model is more complex because chemicals act through many more initiative events than the thyroid hormone receptor. Nevertheless, alternative thyroid assays have been developed, are being validated against reference chemicals, and work will shortly begin on developing a predictive model. Putting these advancements into practice, EDSP has made significant strides in screening large numbers of substances to evaluate possible endocrine effects, as well as narrowing the list of substances in EDSP's chemical universe for which screening or testing may be needed. Since the FY15 report to Congress, over 1800 chemicals have been screened using high throughput assays and computational models to detect potential disruption of the estrogen, androgen, steroidogenesis and thyroid-related pathways of the endocrine system of humans and wildlife.

New Approaches in Development

- Significant work also has been undertaken to develop alternative approaches to assess acute and developmental neurotoxicity. We currently have a moderate to high throughput method, that utilizes neuronal cells in a 96-well format with each well equipped with 16 electrodes. The effects of chemical exposure can be measured on cellular electrical activity. The method to assess acute neurotoxicity has been validated using reference chemicals. The method to assess developmental neurotoxicity shows substantial promise but is still under development.
- The growing use of modeling approaches for screening and data gap filling is becoming an internationally recognized alternative to traditional animal testing. Models that predict physico-chemical properties and environmental fate endpoints are important for understanding the persistence of chemicals in the environment and potential accumulation in different parts of the food web. In 2018, ORD developed the OPERA, or OPEn structure-activity/property Relationship Application, that provides reliable predictions for both physicochemical properties and environmental fate/persistence endpoints³. Modeling and performance details are freely available and have been validated by the European Commission's Joint Research Centre to be compliant with OECD principles for such models. Similarly, predictions of potential toxicity in mammals and ecological species identify the doses or concentrations in the environment that may lead to adverse effects.

² <https://www.epa.gov/endocrine-disruption>

³ Mansouri et al., 2018 DOI:[10.1186/s13321-018-0263-1](https://doi.org/10.1186/s13321-018-0263-1)

- While large amounts of toxicological data are available for some species, data for numerous other plants and animals are very limited. These data are essential for estimating the potential ecological and environmental impacts of chemical exposures. To address this data gap, ORD developed Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS), an online, open-source tool for extrapolating toxicity information across species⁴. Leveraging existing chemical safety information, SeqAPASS evaluates similarities in the proteins that are the targets of certain environmental toxicant across multiple species. SeqAPASS was released internally for testing in August 2014. The first version was released to the public in March 2016 with subsequent updates in May 2017 (v2.0), March 2018 (v3.0) and September 2018 (v3.1).
- One limitation of using high throughput assays to predict toxicity has been the inadequate coverage across all the cellular pathways and processes that may be disrupted by chemicals. In moving forward, several approaches are being implemented to resolve this issue. ORD's computational toxicology effort is testing a new approach to high throughput hazard identification and characterization that directly addresses this limitation. The aim is to cast the broadest net possible for capturing the potential hazards associated with chemical treatment. ORD is applying new technologies that were developed during the human genome project and refined in the commercial sector. The new technologies measure the expression of all the genes in the genome in a high throughput, automatable assay that works directly on a wide variety of human cells⁵. A second approach leverages improvement in automated imaging technologies to measure microscopic changes in human cells⁶. Following treatment with chemicals, the cells are stained with multiple dyes that measure the effects on subcellular organelles and structural features. The automated imaging assay is also high throughput and automatable.

Websites of interest:

- Toxicity Forecaster (ToxCast): <https://www.epa.gov/chemical-research/exploring-toxcast-data-downloadable-data>
- Exposure Forecasting (ExpoCast): <https://www.epa.gov/chemical-research/rapid-chemical-exposure-and-dose-research>
- Chemical and Products Database (CPDat): <https://www.epa.gov/chemical-research/chemical-and-products-database-cpdat>
- CompTox Chemicals Dashboard: <https://comptox.epa.gov/dashboard>
- CompTox Downloadable Data: <https://www.epa.gov/chemical-research/downloadable-computational-toxicology-data>
- SeqAPASS v3.1: <https://seqapass.epa.gov/seqapass/>

Measures of success:

EPA ORD tracks and measures the success of increasing the use of alternatives to animal testing. As new alternative methods are developed, EPA ORD tracks the scientific community's feedback on these approaches. Before using these new approaches to inform decisions, the regulatory

⁴ <https://www.epa.gov/chemical-research/sequence-alignment-predict-across-species-susceptibility>

⁵ Yeakley et al., 2017; DOI:10.1371/journal.pone.0178302

⁶ Bray et al., 2016 DOI:10.1038/nprot.2016.105

community and industry looks to the scientific community to review scientific soundness of these approaches. EPA tracks the scientific community's feedback on scientific products developed by EPA researchers include scientific papers, publications, online tools, data, computer code and software packages. Below is a summary of how EPA ORD tracks the scientific community's use of these new approaches.

- EPA tracks the number of papers published, the number of times a given paper has been cited, the journal impact factor of a paper and the number of times a given paper has been used (read, downloaded). For example, the 2017 paper titled *Public Health Perspective on 21st Century Risk Assessment* authored by Gwinn et al. has been cited 10 times and has over 6,000 abstract views.
- EPA tracks the use of online tools, data, code and software through Google Analytics. The number of new users, returning users and general information about the types of users is collected. For example, in 2017 EPA's online CompTox Chemicals Dashboard had over 150,000 users. EPA's CompTox Chemicals Dashboard is the one-stop online resources for the public to access the data generated from using new approach methods to evaluate chemicals.

In addition to review and acceptance by the scientific community, the use of these methods to inform decisions made about chemicals such as those by EPA, other Federal Agencies, State Agencies, Industry, and International groups can also be tracked.

- EPA ORD is piloting an effort to aggregate policy-related documents that cite or use information generated through EPA's new approach methods. For example, Dupont has used EPA data to register chemicals in Europe and Health Canada uses data to inform the selection of chemicals in its Chemical Management Plan for chemicals with little to no data.
- EPA ORD has hundreds of collaborative research agreements with industry, states, academia, other federal agencies and types of stakeholder groups. Working with these collaborators, EPA ORD is exploring ways to use new approach methods and resulting data. For example, EPA ORD is collaborating with the Unilever (a consumer products company) to use new approach methods to evaluate chemicals in the products they develop. –A complete list of the collaborative agreements are available online at: <https://www.epa.gov/chemical-research/collaborative-agreements-computational-toxicology-research>

From our program partners:

- Refer to OPP's internal efforts to track the reduction in animal use from alternatives.
- Refer to the TSCA alternatives strategic plan for evaluating the potential effects.

See attachment: Final LOREAL EPA CRADA Report.pdf

7. 2015 Report to Congress – language from report that point out the metrics about the use of alternatives for the Endocrine Disruptor Program.

ORD Response: See attachment: Report to Congress FY13-14.pdf

8. Number of research grant reports related to alternatives to animal research from FY16-18.

ORD Response: Currently, NCER does not have grants reporting on alternatives to animal testing. ORD anticipates awarding 5 grants in FY2019 with a focus on research that will promote the development and use of alternative test methods and strategies that address the

“3Rs” of toxicity testing: 1) reduce, 2) refine, and/or 3) replace vertebrate animal testing. For more information see the Request for Applications (RFA) [Advancing Actionable Alternatives to Vertebrate Animal Testing for Chemical Safety Assessment](https://cfpub.epa.gov/ncer_abstracts/index.cfm/fuseaction/display.rfatext/rfa_id/642) (https://cfpub.epa.gov/ncer_abstracts/index.cfm/fuseaction/display.rfatext/rfa_id/642)

9. Animal Subjects Term and Condition (T&C) for EPA Assistance Agreements (grants, cooperative agreements)

ORD Response: The following language is included routinely under the Animal Subjects Term and Condition:

Animal Subjects

The recipient agrees to comply with the Animal Welfare Act of 1966 (P.L. 89-544), as amended, 7 USC 2131-2156. Recipient also agrees to abide by the “U.S. Government Principles for the Utilization and Care of Vertebrate Animals used in Testing, Research, and Training.” (Federal Register 50(97): 20864-20865. May 20,1985). The nine principles can be viewed at <http://grants.nih.gov/grants/olaw/references/phspol.htm#USGovPrinciples>. For additional information about the Principles, the recipient should consult the *Guide for Care and Use of Laboratory Animals*, prepared by the Institute of Laboratory Animal Resources, National Research Council and can be accessed at: <http://www.nap.edu/readingroom/books/labrats/>.

Link to EPA’s general T&Cs: https://www.epa.gov/sites/production/files/2018-09/documents/fy_2019_epa_general_terms_and_conditions_effective_october_1_2018_or_later.pdf

10. Has ORD used the extramural granting process to specifically require or encourage grantees to use alternative methods?

ORD Response: No.

11. Do ORD extramural grant programs ever require researchers to use animal models? If so, please provide examples of why.

ORD Response: In general, the NCER extramural grants program (STAR) does not require researchers to use animal models. However, the “Advancing Actionable Alternatives to Vertebrate Animal Testing for Chemical Safety Assessment” solicitation (see ORD Response 4c) specifies that “although some studies with vertebrates may be needed to validate and establish the relevance, predictive utility, and/or scientific quality of the alternative approach or strategy proposed, use of vertebrate animals in the research should be minimized and existing vertebrate test data should be leveraged to achieve the research objectives wherever scientifically feasible.”